CELL DISRUPTION

Aim: The aim of the experiment is to extract intracellular metabolites to extracellular place by lysing of cells with machanical or non-mechanical (physical, chemical and enzymatical) methods. It is determined and compared the performances of each method by calculating the metabolite concentration.

1. General Information

Many biotechnologically produced compounds are intracellular and must be released from cells before recovery. The efficient recovery of products requires cell disruption, which can be achieved by using different methods and technologies, either mechanical or non-mechanical methods. The chosen technology depends on the product, cell type and scale. Cell disruption is an essential part of biotechnology and the downstream processes related to the manufacturing of biological products. The disruption of cells is necessary for the extraction and retrieval of the desired products, as cell disruption significantly enhances the recovery of biological products.

Generated product either is secreted outer of the cell naturally or stores in the cell. If the desired product is synthesized intracellular, the cell wall should be distrupted with various methods to regain valuable metabolites. During cell distruption processes, valuable metabolites have not to be damaged. It is known the quality and type of distrupted cell well.

Cell Wall: The cell walls of prokaryotes, fungi, algae, and plants are distinctive from each other in chemical composition and microscopic structure, yet they all serve two common primary functions: regulating cell volume and determining cell shape.

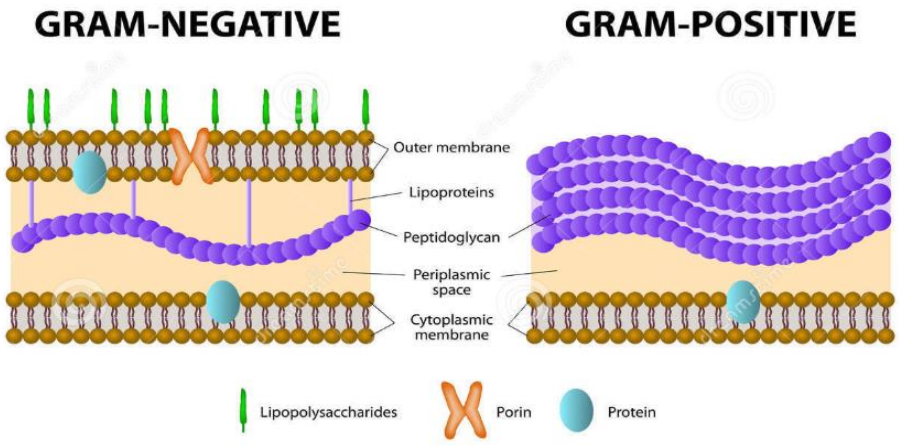

Figure 1: Cell wall of gram negative and gram positive bacteria

Layers which are formed the cell wall have some functions. Outer membrane provide mechanical endurance whereas peptidoglycan layer supplies permeability. Eucaryotic cell wall is more complex than procaryotic cell wall. The main purpose of cell distruption is to distrupt the outer membrane and peptidoglycan layer.

2. Cell Disruption Methods

Cell disruption is an essential part of biotechnology and the downstream processes related to the manufacturing of biological products. The disruption of cells is necessary for the extraction and retrieval of the desired products, as cell disruption significantly enhances the recovery of biological products. Several types of cell disruption methods exist, as biological products may be extracellular, intracellular or periplasmic. Cell disruption methods can be categorised into mechanical methods and non-mechanical methods (Figure 2). Non-mechanical methods can also be divided into physical methods, chemical methods and enzymatic methods.

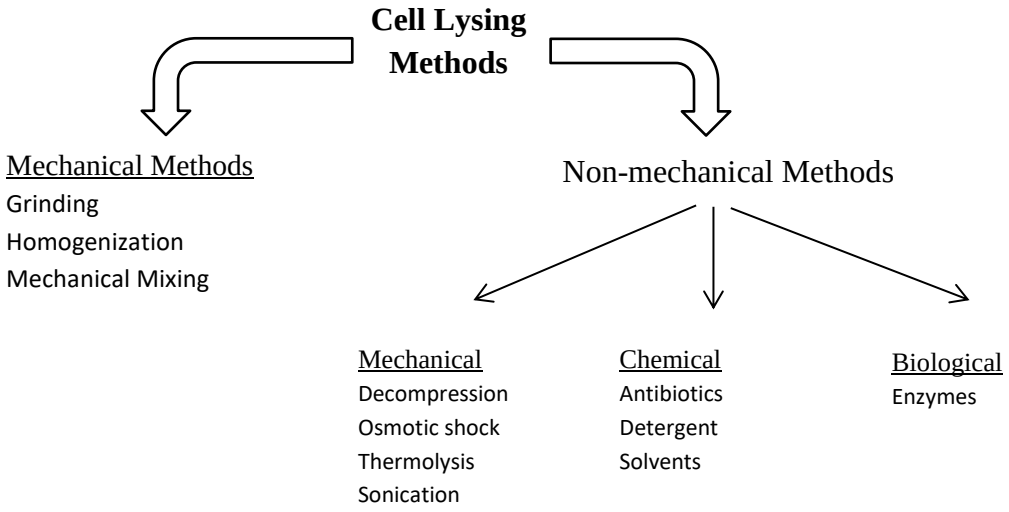

Figure 2: Cell distruption methods

2.1. Mechanical Methods

The main principle of the mechanical disruption methods, is that the cells are being subjected to high stress via pressure, abrasion with rapid agitation with beads, or ultrasound. Some methods of disruption are cavitation, shearing, impingement, or combination of those. Intensive cooling of the suspension after the treatment is required in order to remove the heat that was generated by the dissipation of the mechanical energy.

2.1.1. Grinder (Bead mill)

Bead mills have been originally used in the paint industry, and have been adapted for cell disruption in both small scale and large scale production. It is an efficient way of disrupting different microbial cells as different designs have been developed. The main principle requires a jacketed grinding chamber with a rotating shaft, running in its center (Figure 3). Agitators are fitted with the shaft, and provide kinetic energy to the small beads that are present in the chamber. That makes the beads collide with each other. The choice of bead size and weight is greatly dependent on the type of cells. The diameter can affect the efficiency of

cell disruption in relation of the location of the desired enzyme in the cell. The increased number of beads increases the degree of disruption, due to the increased bead-to-bead interaction. The increased number of beads, however, also affects the heating and power consumption. The process variables are: agitator speed, proportion of the beads, beads size, cell suspension concentration, cell suspension flow rate, and agitator disc design.

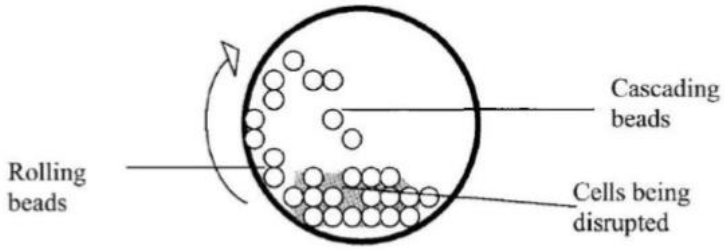

Figure 3: The basic principle of a bead mill

2.1.2. High-pressure homogenization

High-pressure homogenization (HPH) is one of the most widely used mechanical cell disruption methods. High pressure forces the cells through a narrow hole or a channel, which creates a shear force to the cells. Upon discharge from the channel, the cells expand instantly. This instant expansion after the shear force disrupts the cells. A picture of a typical high-pressure homogenizer can be seen in figure 4. The cells can be run through the homogenizer multiple times to ensure total lysis. HPH-method is fast, continuous and easy, but it can have disruptive effects on heat sensitive products, such as enzymes and proteins, because of the temperature increase. High-pressure homogenization is routinely used in pharmaceutical applications for disrupting and for releasing valuable metabolites.

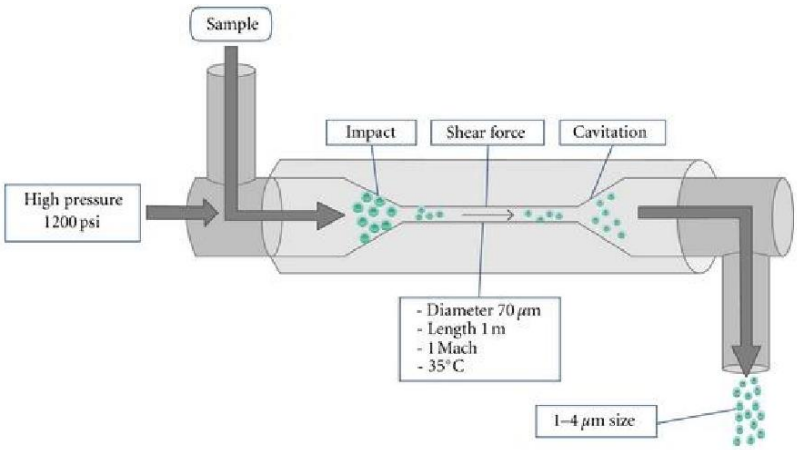

Figure 4: A typical high-pressure homogenizer.

2.1.3. Mechanical Mixing

Blending of blades is achieved by rapid rotation. The cell size should be large. Therefore, it is used in the breakdown of animal and plant cells.

2.2. Non-mechanical Methods

2.2.1. Physical Disruption

Decompression, osmotik shock and termolysis are the methods that have potential for large scale application. Also, sonication method is generally used for laboratory scale.

2.2.1.1. Decompression

In this process, cell suspension is treated with subcritical or supercritical gas under pressure for a length of time. Gas enters into cell, the cell expands with applied pressure and causes disruption during release. This method can be used in yeasts and valuable components can be recovered through supercritical CO_2 extraction. This effective method allows cleaning the cell wastes easily when valuable product is soluble with solvents. But the system works with low productivity. Results are directly proportional with decrease rate of pressure and contact time of gas and suspension.

2.2.1.2. Osmotic Shock

In osmotic shock method cells firstly equilibrate under high osmotic pressure and then medium are diluted suddenly. Water enters into the cell rapidly and causes lysis with rising the internal pressure. The method is especially applied to the cells have weakened wall. But it is ineffective for cells have peptidoglycan which is complex component. When proper osmotic shock is applied to *E. coli* cells, proteins release without any cell viability effects. The system is generally used in small scale due to the high costs of chemicals and high oxygen requirement of wastes.

2.2.1.3.Thermolysis

Thermolysis is the process of cell disruption with high temperature. The outcomes depend on type of organism and growth stage. There are some advantages of this method. Recombinant organisms could not be released into environment and mixed with seperation because the cell is dead. Beside, there are some disadvantages. Liquid can convert into non-Newtonian viscoelastic structure with increasing the viscosity.

2.2.1.4. Sonication

Sonication is especially preferred for small scale applications. Sonicator device produces sonic sound waves inside liquid. These sound waves provide micro scale bubble formation and burst. With releasing energy shock waves disrupt the cell membrane. During the sonication process high energy is absorbed by cell suspension and temperature rises. So, temperature control must be provided in this system. Another point is formation of free radicals. For preventing this, solution must be treated with nitrogen or radical inhibitor compounds must be added into medium.

2.2.2. Chemical Disruption

Many chemical compounds are used for increasing of permeability of cell wall. Solvents generally disrupt membrane with solving hydrophobic part or form pore on the wall with heat. Usage of solvents is not preferred in large scale application for cell disruption and product recovering. Some solvents are toxic and removing of solvents is very costly especially in large biopharmaceutical production. Detergents are very common agents for disruption process, disrupt the cell membrane interaction with water and oil. Detergents especially focus on recovering of membrane proteins. Other important chemical compounds are antibiotics, chelators and hypochlorites for cell disruption.

2.2.3. Biological Disruption

The most used method in biological disruption is enzyme application. Effect of enzymes is specific. Yeasts could be disrupted with stranger lytic enzymes. While protease and glucanase disrupt the glucans of cell wall, lysozyme affects peptidoglycan layers. Also cellulase affects the cell wall of algae.

3. Experiment

3.1. Disruption with vortex

10 mg of dry cell and 10 ml of distilled water are put into 20 ml of glass tubes. 1 mm diameter of glass beads are added into tubes in 50% of tube volume. Tubes are vortexed in 1650 rpm during 2 minutes. Glass beads are removed from the disrupted cells. Finally, water is removed through centrifugation with 3500 rpm during 5 minutes.

3.2. Homogenizer

10 mg of dry cell and 10 ml of distilled water are put into 20 ml of glass tubes. Piston is immersed into the tubes. Disruption process is applied total 2 minutes under the conditions of 20000 rpm and 30 seconds*4 periods. There are 5 seconds between the periods. Then, water is removed through centrifugation with 3500 rpm during 5 minutes.

3.3. Heating with solvent

10 mg of dry cell reacts with 10 ml 40% (v/v) of acetone solvent in water-bath in 80 C° during 2 minutes. Then, biomass is kept in acetone solvent in 4 C° during 24 hours. Finally, acetone is removed through centrifugation with 3500 rpm during 5 minutes.

3.4. Alkali hydrolysis

10 mg of dry biomass is treated with 0.1 M NaOH solution during 15 or 30 minutes. For seperating the disrupted cell from solution, washing process is done for two times and water is removed through centrifugation with 3500 rpm during 5 minutes.

3.5. Disruption with enzyme

Enzyme reaction mixture (10 mg of biomass, 10 mg of cellulase enzyme and 10 ml of distilled water) is incubated in 37 C° of water-bath during 24 hours. Then, washing process is done for two times and medium is removed through centrifugation with 3500 rpm during 5 minutes.

3.6. Sonication

10 mg of dry cell and 10 ml of distilled water are put into 20 ml of glass tubes. Probe of sonicator is immersed into the tubes with caring not touching edge of tubes. For decreasing the effect of heat forming due to sonication process, tubes are put into ice. Sonicator works in 180 W of output power with sending 20 KHz frequency of sound wave during 10 minutes (interval of 0.1 seconds). Finally, centrifugation is done with 3500 rpm during 5 minutes.

3.7. Determining the Performance of Cell Disruption

The performance of cell disruption method is evaluated from microscopic observation or metabolite concentration calculation with organic solvent extraction.

Chlorophyll-a Analysis

5 ml culture is filtered with GF/C paper and 5 ml of methanol is added into filtrate. For chlorophyll-a extraction, extract is kept in 70 C° of water-bath during 2 minutes. Cell wastes are removed through centrifugation with 5000 rpm during 5 minutes. After that, absorbance value of supernatant is obtained in 665 and 750 nm of spectrometer. Chlorophyll-a value is calculated from this formula:

$$\text{Chlorophyll-a (mg/L)} = 13{,}9 \times (A_{665} - A_{750})$$

4. References

Bury, D., Jelen, P., Kallab, M., 2001, Druption of Lactobacillus sp. cells for lactose hydrolysis in dairy products, Food science and emerging Technologies, 2(1):23-29.

Chisti, Y., Moo Young, M., 1986, Disruption of microbial cells for intracellular products, Enzyme microbiology and biotechnology, 8:194-204.

Engler, C.R., 1985, Disruption of microbial cells in comprehensive biotechnology, In: Moo-Young, 2:305-324.

Hetherigton, P.J., Folloes, M., Lilly, M.D., 1971, Release of protein from yeast by homogenizer, Journal of chemical engineers, 49:142-148.

Kobayashi, M., Kurimura, Y., Sakamoto, Y., Tsuji, Y., 1997, Selective extraction of astaxanthin and chlorophyll from green alga Haematococcus pluvialis, Biotech Tech., 11:657-660.

Kula, M.R., Shütte, H., 1987, Purification of proteins and the disruption of microbial cells, Biotechnology Process, 3:31-42.

Lin, H.M., Yang, Z., Chen, L.F., 1992, An improved method for disruption of microbial cells by pressurized CO2, Biotechnol Prog, 8:165-166.

Middelberg, A.P.J., 1995, Process scale disruption of microorganisms, Biotechnol Adv, 13(3): 19-24.